A32 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

98. (Amended) A method for providing a therapeutic G protein coupled receptor signaling modifier peptide to a mammal which

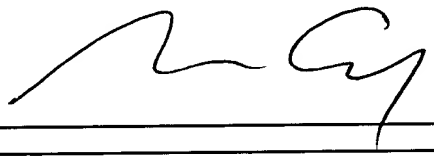
A33 comprises administering to said mammal an expression construct which expresses a peptide according to SEQ ID NOS: 2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

REMARKS

Applicants request that the amendments specified herein and the accompanying initial Sequence Listing be entered prior to examination on the merits. The amendments correct inconsistencies in the sequence identification numbering, very minor typographical errors, and priority information from prior copending provisional application 60/275,472, filed March 14, 2001. The amendments contain no new matter.

Applicants are providing the Sequence Listing in both computer-readable form and paper copy. Applicants hereby state that the information on the computer-readable disk and the paper copy are the same and contain no new matter.

Applicants respectfully request examination on the merits and favorable consideration.

RESPECTFULLY SUBMITTED,			
NAME AND REG. NUMBER	Martha Cassidy Registration No. 44,066		
SIGNATURE		DATE	9/17/01

Address	Rothwell, Figg, Ernst & Manbeck Suite 701-East, 555 13th Street, N.W.				
City	Washington	*State*	D.C.	*Zip Code*	20004
Country	U.S.A.	*Telephone*	202-783-6040	*Fax*	202-783-6031

Attachment: Mark-up of Specification and Claims

Mark-up of the Specification:

On page 6, paragraph 0011:

These [finding] findings have led to a modification of traditional receptor theory (Samama et al., J. Biol. Chem. 268:4625-4636, 1993). It is now thought that receptors can exist in at least two conformations, an inactive conformation (R) and an activated conformation (R*), and that an equilibrium exists between these two states that markedly favors R over R* in the majority of receptors. It has been proposed that in some native receptors and in the mutants described above, there is a shift in equilibrium in the absence of agonist that allows a sufficient number of receptors to be in the active R* state to initiate signaling.

On page 11, paragraph 0020:

The invention provides, in yet a further embodiment a compound selected from the group consisting of SEQ ID NOS:[14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46-105, 115-132 and 147-305] 2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

On page 11, paragraph 0021:

In yet a further embodiment, the invention provides a method for providing a therapeutic G protein coupled receptor signaling modifier peptide to a mammal which comprises administering to said mammal an expression construct which expresses a peptide according to SEQ ID NOS:[14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46-105, 115-132 and 147-305] 2, 4, 6, 8, 10, 12, 13,

15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

On page 12, paragraph 0028:

Figure 7 is a bar graph showing competitive inhibition of high affinity peptides to rhodopsin by [heterotrimenic] heterotrimeric Gt.

On page 12, paragraph 0029:

Figure 8 presents ELISA results from panning CHO cells overexpressing human thrombin receptor (PAR1) using purified MBP-C-terminal fusion proteins. MBP-G11 = xxxx (SEQ ID NO: 1) LQLNLKEYNLV (SEQ ID NO: 2); PAR-13 = VRPS (SEQ ID NO: 3) LQLNRNEYYLV (SEQ ID NO: 4); PAR-23 = LSRS (SEQ ID NO: 5) LQQKLKEYSLV (SEQ ID NO:6); PAR-33 = LSTN (SEQ ID NO: 7) LHLNLKEYNLV (SEQ ID NO: 8); PAR-34 = LPQM (SEQ ID NO: 9) QRLNVGEYNLV (SEQ ID NO: 10); PAR-45 = SRHT (SEQ ID NO: 11) LRLNGKELNLV (SEQ ID NO:[194]12).

Table I, bridging pages 22 and 23:

Table I. Example for Construction of a Synthetic Peptide Library.

```
                          Q  R  M  H  L  R  Q  Y  E  L  L (SEQ ID NO:13)
gaggtggt nnknnknnknnk attcgtgaaaacttaaaagattgtggtcgtttc taa ctaagtaaagc
  A          B                        C                    D      E
```

(SEQ ID NO:[12]14) n = any [amino acid]nucleotide base; k = guanidine or thymidine; A = restriction enzyme site; B = linker sequence; C = oligonucleotide encoding peptide sequence [SEQ ID NO:13]; D = stop codon; E = restriction enzyme site.

On page 23, Table II:

Table II. Gα Subunit Peptides and Corresponding DNA Constructs.

Gα Subunit	Sequence											SEQ ID NO:
Gt	I atc	K aag	E gag	N aac	L ctg	K aaa	D gac	C tgc	G ggc	L ctc	F ttc	[14]15 [15]16
Gi1/2	I ata	K aaa	N aat	N aat	L cta	K aaa	D gat	C tgt	G ggt	L ctc	F ttc	[16]17 [17]18
GRi1/2	N aac	G ggc	I atc	K aag	C tgc	L ctc	F ttc	N aac	D gac	K aag	L ctg	[18]19 [19]20
Gi3	I att	K aaa	N aac	N aac	L tta	K aag	E gaa	C tgt	G gga	L ctt	Y tat	[20]21 [21]22
Go2	I atc	A gcc	K aaa	N aac	L ctg	R cgg	G ggc	C tgt	G gga	L ctc	Y tac	[22]23 [23]24
Go1	I att	A gcc	N aac	N aac	L ctc	R cgg	G ggc	C tgc	G ggc	L ttg	Y tac	[24]25 [25]26
Gz	I ata	Q cag	N aac	N aat	L ctc	K aag	Y tac	I att	G ggc	L ctt	C tgc	[26]27 [27]28
G11	L ctg	Q cag	L ctg	N aac	L ctc	K aag	E gag	Y tac	N aac	L ctg	V gtc	[28]2 29
Gq	L ctc	Q cag	L ttg	N aac	L ctg	K aag	E gag	Y tac	N aat	A gca	V gtc	30 31
Golf	Q cag	R cgg	M atg	H cac	L ctc	K aag	Q cag	Y tat	E gag	L ctc	L ttg	32 33
G14	L cta	Q cag	L cta	N aac	L cta	R agg	E gaa	F ttc	N aac	L ctt	V gtc	34 35
G15/16	L ctc	A gcc	R cgc	Y tac	L ctg	D gac	E gag	I atc	N aac	L ctg	L ctg	36 37
G12	L ctg	Q cag	E gag	N aac	L ctg	K aag	D gac	I atc	M atg	L ctg	Q cag	38 39
G13	L ctg	H cat	D gac	N aac	L ctc	K aag	Q cag	L ctt	M atg	L cta	Q cag	40 41
Gs	Q cag	R cgc	M atg	H cac	L ctt	R cgt	Q cag	Y tac	E gag	L ctg	L ctc	[42]13 [43]42
5' - gatccgccgccaccatggga-												-tgaa-3'

(SEQ ID NOS:[44]43, [45]44)

On pages 24-25, Table III:

Table III. Exemplary Native G Protein Sequences for Library or Minigene Construction.*

Name	Sequence	SEQ ID NO:	Name	Sequence	SEQ ID NO:
[hgt]hGt	IKENLKDCGLF	[46]15	CryptoGba1	LQNALRDSGIL	[76]62
hGi1/2	IKNNLKDCGLF	[47]17	GA3_UST	LTNALKDSGIL	[77]63
G05_DRO	IKNNLKQIGLF	[48]45	GA1_KLU	IQQNLKKSGIL	[78]64
GAF_DRO	LSENVSSMGLF	[49]46	GA3_UST	LTNALKDSGIL	[79]63
Gi-DRO	IKNNLKQIGLF	[50]45	GA1_DIC	NLTLGEAGMIL	[80]64
hGi3	IKNNLKECGLY	[51]21	GA2_KLU	LENSLKDSGVL	[81]65
hGO-1	IANNLRGCGLY	[52]25	GA2_UST	ILTNNLRDIVL	[82]66
hGO-2	IAKNLRGCGLY	[53]47	Mgs-XL	QRMHLPQYELL	[83]67
GAK_CAV	IKNNLKECGLY	[54]21	hGs	QRMHLRQYELL	[84]13
G0_XEN	IAYNLRGCGLY	[55]48	hGolf	QRMHLKGYELL	[85]68
GA3_CAEEL	IQANLQGCGLY	[56]49	GA1_COPCO	LQLHLRECGLL	[86]69
GA2_CAEEL	IQSNLHKSGLY	[57]50	GA1-SOL	RRRNLFEAGLL	[87]70
GA1_CAEEL	LSTKLKGCGLY	[58]51	GA2_SB	RRRNLLEAGLL	[88]71
GAK_XEN	IKSNLMECGLY	[59]52	GA1_SB	RRRNPLEAGLL	[89]72
GA1_CAN	VQQNLKKSGIM	[60]53	GA1_UST	IQVNLRDCGLL	[90]73
hGZ	IQNNLKYIGLC	[61]27	GA4_UST	RENLKLTGLVG	[91]74
hG15	LARYLDEINLL	[62]26	GA1_ORYSA	DESMRRSREGT	[92]75
GA2_SCHPO	LQHSLKEAGMF	[63]54	GQ1_DROME	MQNALKEFNLG	[93]76
hG12	LQENLKDIMLQ	[64]38	GA2_DIC	TQCVMKAGLYS	[94]77
hG13	LHDNLKQLMLQ	[65]40	GS-SCH	LQHSLKEAGMF	[95]54
GAL_DRO	LQRNLNALMLQ	[66]55	GA-SAC	ENTLKDSGVLQ	[96]56
GA2_YST	ENTLKDSGVLQ	[67]56	GA1-CE	IISASLKMVGV	[97]78
hG14	LQLNLREFNLV	[68]34	GA2-CE	NENLRSAGLHE	[98]79
hG11	LQLNLKEYNLV	[69]2	GA3-CE	RLIRYANNIPV	[99]80
hGQ	LQLNLKEYNAV	[70]30	GA4-CE	LSTKLKGCGLY	[100]51
GQ_DROME	LQSNLKEYNLV	[71]57	GA5-CE	IAKNLKSMGLC	[101]81
G11_XEN	LQHNLKEYNLV	[72]58	GA6-CE	IGRNLRGTGME	[102]82
Gq_SPOSC	IQENLRLCGLI	[73]59	GA7-CE	IQHTMQKVGIQ	[103]83
GA1_YST	IQQNLKKIGII	[74]60	GA8-CE	IQKNLQKAGMM	[104]84
GA1_NEUCR	IIQRNLKQLIL	[75]61	GA5-DIC	LKNIFNTIINY	[105]85

*For production of minigene constructs each nucleotide sequence should be constructed to encode the amino acids MG at the N-terminus of the peptide by using 5'-gatccgccgccaccatggga-(SEQ ID NO:[44]43) and -tgaa-3' (SEQ ID NO:[45]44).

On page 29 Table IV:

Table IV. Diversity in Unpanned Gq Library.

		SEQ. ID NO.
Native	LQLNLKEYNLV	[106]2
clone #1	LLLQLVEHTLV	[107]86
clone #2	HRLNLLEYCLV	[108]87
clone #3	EQWNMNTFHMI	[109]88
clone #4	SQVKLQKGHLV	[110]89
clone #5	LRLLL*EYNLG	[111]90
clone #6	RRLKVNEYKLL	[112]91
clone #7	LQLRLREHNLV	[113]92
clone #8	HVLNSKEYNQV	[114]93

On page 30, Table V:

Table V. Selection in Panned Gα11 Library.

		SEQ ID NO.
Native	LQLNLKEYNLV	[106]2
Round 1		
1	MKLNVSESNLV	[115]94
2	LQTNQKEYDMD	[116]95
3	LQLNPREDKLW	[117]96
4	RHLDLNACNMG	[118]97
5	LR*NDIEALLV	[119]98
6	LVQDRQESILV	[120]99
Round 2		
1	LQLKHKENNLM	[121]100
2	LQVNLEEYHLV	[122]101
3	LQFNLNDCNLV	[123]102
4	MKLKLKEDNLV	[124]103
5	HQLDLLEYNLG	[125]104
6	LRLDFSEKQLV	[126]105
Round 3		
1	LQKNLKEYNMV	[127]106
2	LQYNLMEDYLN	[128]107
3	LQMYLRGYNLV	[129]108
4	LPLNPKEYSLV	[130]109
5	MNLTLKECNLV	[131]110
6	LQQSLIEYNLL	[132]111

On page 43 Table VI:

Table VI. Exemplary Sequences of C-terminal Minigene Peptides.

Peptide Name	Sequence	SEQ ID NO:
Gαi	MGIKNNLKDCGLF	[133]112
GαiR	MGNGIKCLFNDKL	[134]113
Gαq	MGLQLNLKEYNAV	[135]114
Gαq**	MGLQLNLKEYNTL	[136]115
Gα12	MGLQENLKDIMLQ	[137]116
Gα13	MGLHDNLKQLMLQ	[138]117

Paragraph 0100, bridging pages 49 and 50:

Construction of a biased peptide library has been described previously. Martin et al., *J. Biol. Chem.* 271:361-366, 1996; Schatz et al., *Meth. Enzymol.* 267:171-191, 1996. The vector used for library construction was pJS142 (see Figure 2). This vector had a linker sequence between the LacI and the biased [undercamer] undecamer peptide coding sequence, as well as restriction sites for cloning the library oligonucleotide. The oligonucleotide synthesized to encode the mutagenesis library was synthesized with 70% of the correct base and 10% of each of the other bases at each position. This mutagenesis rate leads to a biased library such that there is approximately a 50% chance that any of the 11 codons will be the appropriate amino acid and approximately a 50% chance that it will be another amino acid. In addition, a linker of four random NNK (where N denotes A, C, G or T and K denotes G or T) codons were synthesized at the 5' end of the sequence to make a total of 15 randomized codons. Using this method, a library with greater than 10^9 independent clones

per microgram of vector used in the ligation was constructed based on the carboxyl terminal sequence of Gαt (IKENLKDCGLF; SEQ ID NO:[139]15). The nucleic acid used for creating this library was: [5'-]

5'-GAGGTGGTNNKNNKNNKNNKattcaaggagaacctgaaggactgcggcctcttcTAACTAAGTAAAGC-3', wherein N= A/C/G/T and K= G/T; SEQ ID NO:[140]118).

On page 50, Table VI:

Table [VI]VII. C-Terminal Gα Subunit Peptide Library Constructs.

Gα Sub-unit	RE	Linker	Peptide Coding Region	Stop	RE	SEQ ID NO:
Gs	5-GAGGTGGT	NNKNNKNNKNNK	attcgtgaaaacttaaaagattgtggtcgtttc	TAA	CTAAGTAAAGC-3'	[141] 14
G11	5-GAGGTGGT	NNKNNKNNKNNK	ctgcagctgaacctgaaggagtacaatctggtc	TAA	CTAAGTAAAGC-3'	[142] 119
G12	5-GAGGTGGT	NNKNNKNNKNNK	ctgcaggagaacctgaaggacatcatgctgcag	TAA	CTAAGTAAAGC-3'	[143] 120
G13	5-GAGGTGGT	NNKNNKNNKNNK	ctgcatgacaacctcaagcagcttatgctacag	TAA	CTAAGTAAAGC-3'	[144] 121
G15	5-GAGGTGGT	NNKNNKNNKNNK	ctcgcccggtacctggacgagattaatctgctg	TAA	CTAAGTAAAGC-3'	[145] 122
Gz	5-GAGGTGGT	NNKNNKNNKNNK	atacagaacaatctcaagtacattggcctttgc	TAA	CTAAGTAAAGC-3'	[146] 123

On page 58, paragraph 0114:

The panning process is illustrated in Figure 1. For screening of the library by "panning," rhodopsin receptors prepared according to Example 5 were immobilized directly on Immulon 4 (Dynatech) microtiter wells (0.1-1 µg of protein per well) in cold 35 MM HEPES, pH 7.5, containing 0.1 mM EDTA, 50 mM KCl and 1mM [dithiothrietol]dithiothreitol (HEK/DTT). After shaking for one hour at 4°C, unbound membrane fragments were washed away with HEK/DTT. The wells were blocked with 100 µl 2% BSA in HEKL (35 [Mm] mM HEPES; [0.1 mM]0.1 mM EDTA; 50 mM KCl; 0.2 M α-lactose; pH 7.5, with 1 mM DTT). For rounds 1 and 2, BSA was used for blocking; in later rounds 1% nonfat dry milk was used. For the first round of panning, about 24 wells of a 96-well plate were used. In subsequent rounds, 8 wells with receptor and 8 wells without receptor were prepared.

On page 61, Table IX:

Table IX. Light-Activated Rhodopsin High Affinity Sequences.

Clone No.	SEQ ID NO:	Sequence
Library Sequence	[139]124	[I R E N L K D C G L F] IRENLKDCGLF
8	[147]125	[L L E N L R D C G M F] LLENLRDCGMF
9	[148]126	[I Q G V L K D C G L L] IQGVLKDCGLL
10	[149]127	[I C E N L K E C G L F] ICENLKECGLF
18	[150]128	[M L E N L K D C G L F] MLENLKDCGLF
23	[151]129	[V L E D L K S C G L F] VLEDLKSCGLF
24	[152]130	[M L K N L K D C G M F] MLKNLKDCGMF
3	[153]131	[L L D N I K D C G L F] LLDNIKDCGLF
4	[154]132	[I L T K L T D C G L F] ILTKLTDCGLF
6	[155]133	[L R E S L K Q C G L F] LRESLKQCGLF
11	[156]134	[I H A S L R D C G L F] IHASLRDCGLF
13	[157]135	[I R G S L K D C G L F] IRGSLKDCGLF
14	[158]136	[I F L N L K D C G L F] IFLNLKDCGLF
15/28	[159]137	[I R E N L E D C G L F] IRENLEDCGLF
16	[160]138	[I I D N L K D C G L F] IIDNLKDCGLF
17	[161]139	[M R E S L K D C G L F] MRESLKDCGLF
19	[162]140	[I R E T L K D C G L L] IRETLKDCGLL
26	[163]141	[I L A D V I D C G L F] ILADVIDCGLF
27	[164]142	[M C E S L K E C G L F] MCESLKECGLF

On page 62, Table X:

Table X. Dark-Adapted Rhodopsin High Affinity Sequences.

Clone No.	SEQ ID NO:	Sequence
Library Sequence	[139]124	[I R E N L K D C G L F] IRENLKDCGLF
2	[165]143	[I R E K W K D L A L F] IREKWKDLALF
3	[166]144	[V R D N L K N C F L F] VRDNLKNCFLF
7	[167]145	[I G E Q I E D C G P F] IGEQIEDCGPF
17	[168]146	[I R N N L K R Y G M F] IRNNLKRYGMF
21	[169]147	[I R E N L K D L G L V] IRENLKDLGLV
26	[170]148	[I R E N F K Y L G L W] IRENFKYLGLW
33/37	[171]149	[S L E I L K D W G L F] SLEILKDWGLF
41	[172]150	[I R G T L K G W G L F] IRGTLKGWGLF

On page 62, paragraph 0118:

The methods of Example 7 were used to screen different sources of PAR1 receptor using the Gq library. Purified PAR1, reconstituted in lipid vesicles (Example 6), membranes prepared from Sf9 insect cells expressing PAR1 (Example 2) and membranes prepared from mammalian cells overexpressing PAR1 were used. The results of the screens are presented in Tables XI, XII and XIII, respectively. The peptide used as the competitor was LQLNLKEYNLV (SEQ ID NO:[56]2).

On Page 63, Table XI:

Table XI. Reconstituted Purified Recombinant PAR1 Receptor; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: [69]2
R2-16	*SWV	[319]151	LQFNLNDCNLV	[173]102
R2-17	FVNC	[320]152	LQRNKKQYNLG	[174]160
R2-18	EVRR	[321]153	MKLKLKEDNLV	[175]103
R2-20	*RVQ	[322]154	HQLDLLEYNLG	[176]104
R2-21	RLTR	[323]155	LQLRYKCYNLV	[177]161
R3-37	SR*K	[324]156	LQQSLIEYNLL	[178]111
R3-38	MTHS	[325]157	VHVKLKEYNLV	[179]162
R3-44	SGPQ	[326]158	LQLNVKEYNLV	[180]163
R3-46	ML*N	[327]159	LRIYLKGYNLV	[181]164

On Page 63, Table XII:

Table XII. PAR1 Receptor Sf9 Insect Cell Membranes; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: 2
S1-13	S*IR	[328]165	MKLNVSESNLV	[182]94
S1-18	RWIV	[329]166	LQLNLKVYNLV	[183]175
S1-23	G*GH	[330]167	LELNLKVYNLF	[184]176
S2-26	RSEV	[331]168	LQLKHKENNLM	[185]100
S2-30	CEPG	[332]169	LHLNMAEVSLV	[186]177
S2-36	HQMA	[333]170	LQVNLEEYHLV	[187]101
S3-6	VPSP	[334]171	LQKNLKEYNMV	[188]106
S3-8	QMPN	[335]172	LQMYLRGYNLV	[189]108
S3-10	MWPS	[336]173	LKRYLKESNLV	[190]178
S3-15	C*VE	[337]174	MNLTLKECNLV	[191]110

On page 63, Table XIII:

Table XIII. Mammalian (CHO) Cells Overexpressing PAR1; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: 2
C4-5	PRQL	[338]179	LQLKRGEYILV	[192]183
C4-19	VRPS	[339]3	LQLNRNEYYLV	[193]3
C5-10	SRHT	[340]11	LRLNGKELNLV	[194]12
C5-12	FFWV	[341]180	CSLKLKAYNLV	[195]184
C4-16	ORDT	[342]181	LQMNHNEYNLV	[196]185
C7-3	NFRN	[343]182	PQLNLNAYNLV	[197]186
C7-10	LPQM	[344]9	QRLNVGEYNLV	[198]10
C7-13	LSTN	[345]7	LHLNLKEYNLV	[199]8
C7-14	LSRS	[346]4	LQQKLKEYSLV	[200]6

On page 64, Table XIV:

Table XIV. β2-Adrenergic Receptor screened with Gs library.

Competitor	QRMHLRQYELL	SEQ ID NO: [84]13	ELISA
AG1	QGMQLRRFKLR	[201]187	.435
AG20	RWLHWQYRGRG	[202]188	.431
AG19	PRPRLLRFKIP	[203]189	.361
AG2	QGEHLRQLQLQ	[204]190	.330
AG4	QRLRLGPDELF	[205]191	.291
BAR1	QRIHRRPFKFF	[206]192	.218
AG3	QRMPLRLFEFL	[207]193	.217
BAR2	QRVHLRQDELL	[208]194	.197
AG11	DRMHLWRFGLL	[209]195	.192
AG9	QRMPLRQYELL	[210]196	.190
BAR3	QWMDLRQHELL	[211]197	.185
AG18	QRMNLGPCGLL	[212]198	.155
BAR20	NCMKFRSCGLF	[213]199	.079
AG13	QRLHLRGYEFL	[214]200	.054
BAR11	HRRHIGPFALL	[215]201	.048
BAR8	ERLHRRLFQLH	[216]202	.047
BAR40	PCIQLGQYESF	[217]203	.028
BAR31	QRLRLRKYRLF	[218]204	.026

On page 65, Table XV:

Table XV. Rhodopsin screened with Gt library.

		SEQ ID NO:	
Competitor	IRENLKDCGLF	[14]124	ELISA
L33	IVEILEDCGLF	[219]205	1.007
L4	MLDNLKACGLF	[220]206	.908
L3	ILENLKDCGLF	[221]207	.839
L14	LRENLKDCGLL	[222]208	.833
L38	LLDILKDCGLF	[223]209	.823
L15	VRDILKDCGLF	[224]210	.621
L34	ILESLNECGLF	[225]211	.603
L17	ILQNLKDCGLF	[226]212	.600
L7	MLDNLKDCGLF	[227]213	.525
L10	IHDRLKDCGLF	[228]214	.506
L20	IRGSLKDCGLF	[229]135	.423
L6	ICENLKDCGLF	[230]215	.342
L8	IVKNLEDCGLF	[231]216	.257
L13	ISKNLRDCGLL	[232]217	.187
L10	IRDNLKDCGLF	[233]218	.162

On page 66, paragraph 0120:

Chinese hamster ovary-expressed PAR1 was screened against the Gt, G12 and G13 libraries, using the competitor peptide indicated in Table XVI below. Additional peptide analogs were identified using the G11 library and LQLNLKEYNLV (SEQ ID NO:[243]2) as competitor and screened for high affinity binding to PAR1 receptor obtained from different sources, indicated in Table XVII, below.

On Page 66, Table XVI:

Table XVI. Peptides Identified with CHO EXPRESSED PAR1.

Gt library (IRENLKDCGLF; SEQ ID NO:[14]124)	G12 library (LQENLKDIMLQ; SEQ ID NO:[64]38)	G13 library (LQDNLKQLMLQ; SEQ ID NO:[65]233)
IREFLTDCGLF [234]219	LQENLKEMMLQ [240]225	LQDNLRHLMLQ [248]234
IRLDLKDVSLF [235]220	LEENLKYRMLD [241]226	LQDKINHLMLQ [249]235
ICERLNDCGLC [236]221	LQEDLKGMTLQ [242]227	LQANRKLGMLQ [250]236
PRDNTKVRGLF [237]222	LQETMKDQSLQ [243]228	LIVKVKQLIWQ [251]237
FWGNLQDSGLF [230]223	PQVNLKSIMRQ [244]229	MRAKLNNLMLE [252]238
RRGNGKDCRHF [239]224	WQHKLSEVMLQ [245]230	LQDNLRHLIQ [253]239
	LKEHLMERMLQ [246]231	LQDNRNQLLF [254]240
	LLGMLEPLMEQ [247]232	

On page 67 Table XVII:

Table XVII. PAR1 Binding Peptides Screened using a G11 Library (LQLNLKEYNLV; SEQ ID NO: 2)

CHO EXPRESSED	SEQ ID NO:	Recomb/Reconst	SEQ ID NO:	SF9 EXPRESSED	SEQ ID NO:
LQLNVKEYNLV	[255]163	LQLNVKEYNLV	[275]163	LQLNLKVYNLV	[289]175
LQLNRKNYNLV	[256]241	LQLRVKEYKRG	[276]244	LQLKHKENNLM	[290]100
LQLRYKCYNLV	[257]161	LQLRYKCYNLV	[277]161	LQKNLKEYNMV	[291]106
LQLDLKESNMV	[258]242	LQIYLKGYNLV	[278]245	LQVNLEEYHLV	[292]101
LQLNLKKYNRV	[259]243	LQFNLNDCNLV	[279]102	LFLNLKEYSLV	[293]257
LQLRVKEYKRG	[260]244	LQRNKKQYNLG	[280]160	LELNLKVYNLV	[294]258
LQRNKKQYNLG	[261]160	LQRNKNQYNLG	[281]254	LPLNPKEYSLV	[295]109
LQIYLKGYNLV	[262]245	LQQSLIEYNLL	[282]111	LPLNLIDFSLM	[296]259
LQFNLNDCNLV	[263]102	LRLDFSEKQLV	[283]105	LPRNLKEYDLG	[297]260
LQYNLKESFVV	[264]246	LYLDLKEYCLF	[284]255	LRLNDIEALLV	[298]261
LQQSLIEYNLL	[265]111	HQLDLLEYNLG	[285]104	LVLNRIEYNLL	[299]262
LQRDHVEYKLF	[266]247	VQVKLKEYNLV	[286]251	LHLNMAEVSLV	[300]177
LVIKPKEFNLV	[267]248	MKLKLKEDNLV	[287]103	MNLTLKECNLV	[301]110
IQLNLKNYNIV	[268]249	SAKELDQYNLG	[288]256	MKLNVSESNLV	[302]94
HQLDLLEYNLG	[269]104			LKRYLKESNLV	[303]178
MQLNLKEYNLV	[270]250			LKRKLKESNMG	[304]263
VQVKLKEYNLV	[271]251			LKRKVKEYNLG	[305]264
QLLNQYVYNLV	[272]252				
MKLKLKEDNLV	[273]103				
WRLSLKVYNLV	[274]253				

Paragraph 0121, bridging pages 67 and 68:

In the last round of panning, several clones were selected from the (+) receptor plates and grown up overnight in LB-Amp media. Three hundred microliters of the overnight culture was diluted in 3 mL in LB-Amp media for "ELISA lysate culture." Another 30 µL was added to an equal volume of 50% glycerol was stored in labeled microcentrifuge tubes at -70°C. The remaining 4.5 mL was used to make DNA using a standard miniprep protocol

(Qiagen Spinprep™ kits) and sequenced using a 19 base pair reverse primer which is homologous to the vector at a site 56 basepairs downstream from the TAA stop condon that terminates the random region of the library (GAAAATCTTCTCTCATCCG; SEQ ID NO:[306]265). The DNA was stored at -20°C. The ELISA lysate culture was allowed to shake for one hour at 37°C. Expression was induced by adding 33 µL 20% arabinose (0.2% final concentration) with shaking at 37°C for 2-3 hours. The culture then was subjected to sedimentation at 4000 xg for five minutes, the pellet resuspended in 3 mL cold WTEK buffer, resedimented at 4000 xg for five minutes and the pellet resuspended in 1 mL cold TEK buffer. After transfer to 1.5 mL microcentrifuge tubes, the pellet was sedimented at 13,000 xg for two minutes and the supernatant aspirated. The cell pellet was resuspended in 1 mL lysis buffer (42 mL HE, 5 mL 50% glycerol, 3 mL 10 mg/mL BSA in HE, 750 µL 10 mg/mL lysozyme in HE and 62.5 µL 0.2 M PMSF) and incubated on ice for one hour. One hundred ten microliters 2M KCl was added to the lysis mixture and inverted to mix, then sedimented at 13,000 xg for 15 minutes at 4°C. The clear crude lysate (about 0.9 mL supernatant) was transferred to a new tube and stored at -70°C.

Paragraph 0123, bridging pages 69 and 70:

To identify peptides having even higher affinity to light-activated rhodopsin than those identified by the panning procedure described in Example 7, a high affinity peptide was included in the library incubations in rounds three and four.

Peptide 8 (LLENLRDCGMF; SEQ ID NO:[147]125) had been identified in the first screening as a peptide exhibiting binding to light-activated rhodopsin 1000-fold higher than the native sequence. Screening of the Gαt library was performed as in Example 7, except that 10 µL 100 µM (100 nM final concentration) peptide 8 was included in the wells in rounds three and four. This screen revealed several clones that both bind rhodopsin with very high affinity and stabilize it in its active form, metarhodopsin II. See Table XVIII, below. Comparing Tables IX and XVIII, it is clear that the use of peptide 8 in the screen resulted in a change at position 341 to a neutral residue. Residues L344, C347 and G348 remained stable whether peptide 8 was included in the screen or not. Use of peptide 8 resulted in a higher incidence of isoleucine at position 340 (17% with native peptide versus 71% with peptide 8) and a lower incidence of glutamine at position 342 (67% with native peptide versus 29% with peptide 8) This type of information not only contributes to the discovery of highly potent analog peptides for use as drugs or drug screening compounds, but also furthers the understanding of the structural framework which underlies the sites of contact between Gα and receptor.

On page 71, Table XVIII:

Table XVIII. Exemplary Light-Activated Rhodopsin High Affinity Sequences Identified in Screens with Addition of Peptide 8.

Clone No.	SEQ ID NO:	Sequence
Library Sequence	[14]124	[I R E N L K D C G L F] IRENLKDCGLF
Peptide 8	[147]125	[L L E N L R D C G M F] LLENLRDCGMF
3	[307]266	[I L E N L K D C G L L] ILENLKDCGLL
7	[308]213	[M L D N L K D C G L F] MLDNLKDCGLF
8	[309]216	[I V K N L E D C G L F] IVKNLEDCGLF
10	[310]218	[I R D N L K D C G L F] IRDNLKDCGLF
13	[311]217	[I S K N L R D C G L L] ISKNLRDCGLL
17	[312]212	[I L Q N L K D C G L F] ILQNLKDCGLF
19	[313]206	[M L D N L K A C G L F] MLDNLKACGLF

Paragraph 0136, bridging pages 78 and 79:

cDNA encoding the last 11 amino acids of Gα subunits was synthesized (Great American Gene Company) with newly engineered 5'- and 3'- ends. The 5'- end contained a BamHI restriction enzyme site followed by the human ribosome-binding consensus sequence (5'- GCCGCCACC-3'; SEQ ID NO:[314]267), a methionine codon (ATG) for translation initiation, and a glycine codon (GGA) to protect the ribosome binding site during translation and the nascent peptide against proteolytic degradation. A HindIII restriction enzyme site was synthesized at the 3' end immediately following the translational stop codon (TGA). Thus, the full-length 56 bp oligonucleotide for the $Gi\alpha_{1/2}$ carboxyl terminal sequence was

5'-gatccgccgccaccatgggaatcaagaacaacctgaaggactgcggcctcttctgaa -3' (SEQ ID NO:[315]268) and the complimentary strand was 5'-agctttcagaagaggccgcagtccttcaggttgttcttgattcccatggtggcggcg-3' (SEQ ID NO:[316]269). See Figure 11. As a control, oligonucleotides encoding the $G\alpha i_{1/2}$ carboxyl terminus in random order (GαiR) with newly engineered 5'- and 3'- ends also were synthesized. The DNA was diluted in sterile ddH_2O to form a stock concentration at 100 µM. Complimentary DNA was annealed in 1X NEBuffer 3 (50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT; New England Biolabs) at 85°C for 10 min then allowed to cool slowly to room temperature. The DNA then was subjected to 4% agarose gel electrophoresis and the annealed band was excised. DNA was purified from the band using a kit, according to the manufacture's protocol (GeneClean II Kit, Bio101). After digestion with each restriction enzyme, the pcDNA 3.1(-) plasmid vector was subjected to 0.8% agarose gel electrophoresis, the appropriate band cut out, and the DNA purified as above (GeneClean II Kit, Bio101). The annealed/cleaned cDNA was ligated for 1 hour at room temperature into the cut/cleaned pcDNA 3.1 plasmid vector (Invitrogen) previously cut with BamHI and HindIII. For the ligation reaction, several different ratios of insert to vector cDNA (ranging from 25 µM:25 pM to 250 pM:25 pM annealed cDNA) were plated. Following the ligation reaction, the samples were heated to 65°C for 5 min to deactivate the T4 DNA ligase. The ligation mixture (1 µl) was electroporated into 50 µl competent cells as described in Example 7 and the cells

immediately placed into 1 ml of SOC (Gibco). After 1 hour shaking at 37°C, 100 µl of the electroporated cells containing the minigene plasmid DNA was spread on LB/Amp plates and incubated at 37°C for 12-16 hours. To verify that insert was present, colonies were grown overnight in LB/Amp and their plasmid DNA purified (Qiagen SpinKit). The plasmid DNA was digested with NcoI (New England Biolabs, Inc.) for 1 hour at 37°C and subjected to 1.5% (3:1) agarose gel electrophoresis. Vector alone produced 3 bands. When the 56 bp annealed oligonucleotide insert is present, there is a new NcoI site resulting in a shift in the band pattern such that the digest pattern goes from three bands (3345 bp, 1352 bp, 735 bp) to four bands (3345 bp, 1011 bp, 735 bp, 380 bp). See Figure 12. DNA with the correct electrophoresis pattern was sequenced to confirm the appropriate sequence. This method may be used to insert any high affinity peptide to create a minigene constant.

Paragraph 0138, bridging pages 80 and 81:

Human embryonic kidney (HEK) 293 cells were transfected using a standard calcium phosphate procedure according to the methods of Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harpor Laboratory Press, New York, vol. 1-3 (1989), the disclosures of which are hereby incorporated by reference. To confirm the transcription of minigene constructs in transfected cells, total RNA was isolated from the cells 48 hours post transfection with pcDNA-Gαi or pcDNA-GαiR using methods known in

the art. Reverse transcriptase PCR was used to make cDNA and PCR analysis was performed using the cDNA as template with primers specific for the relevant Gα carboxyl terminal peptide insert (forward: 5'-ATCCGCCGCCACCATGGGA (SEQ ID NO:[317]270); reverse: 5'-GCGAAAGGAGCGGGGCGCTA (SEQ ID NO:[318]271). These primers for the Gα minigenes amplify a 434 bp fragment only if the inserted peptide-encoding oligonucleotides are present; no band is observed in cells transfected with the empty pcDNA3.1 vector. The PCR products were separated on 1.5% agarose gels. The presence of a single 434 bp band indicated that Gα carboxyl terminus peptide minigene RNA had been transcribed. See Figure 13. Control experiments were done using a T7 forward primer with the vector reverse primer to verify the presence of the pcDNA3.1 vector, and G3DPH primers (Clonetech) to approximate the amount of total RNA.

In the Claims:

94. (Amended) A compound identified by the method of claim 1, which comprises a peptide selected from the group consisting of SEQ ID NOS: [14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46-105, 115-132 and 147-305] 2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

95. (Amended) A compound selected from the group consisting of SEQ ID NOS: [14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40,

42, 46-105, 115-132 and 147-305] 2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

98. (Amended) A method for providing a therapeutic G protein coupled receptor signaling modifier peptide to a mammal which comprises administering to said mammal an expression construct which expresses a peptide according to SEQ ID NOS: [14, 16, 20, 22, 26, 28, 30, 32, 34, 36, 38, 40, 42, 46-105, 115-132 and 147-305] 2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.